

## Clustering of Macroions in Solutions of Highly Asymmetric Electrolytes

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**ABSTRACT** In this paper, we present results of computer simulations for a primitive model of asymmetric electrolyte solutions containing macroions, counterions and in a few cases, also co-ions. The results show that the valency of counterions plays an important role in shaping the net interaction between the macroions. For solutions with monovalent counterions, the macroions are distributed at larger distances, and in solutions with divalent counterions, the macroions come closer to each other and share a layer of counterions, whereas, in solutions with trivalent counterions, the macroions form clusters. These clusters dissolve upon dilution or addition of a simple electrolyte. These findings suggest a mechanism whereby the nonuniform distribution of macroions observed experimentally in charged systems may occur.

### INTRODUCTION

Suspensions of charged colloids, solutions of surfactant micelles, and globular proteins play an important role in technology and/or biological processes. The problem of the stability of these systems has been considered to be solved to a satisfying degree in the framework of the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (Verwey and Overbeek, 1948). According to this approach, an overlap of the electrical double-layers yields a repulsive interaction, and van der Waals forces are responsible for attraction. Some experimental observations, e.g., the occurrence of large stable voids in homogeneous suspensions (see, for example, Ito et al., 1994), separations into two phases (Tata et al., 1992), and the salt concentration dependence of the interparticle distance in colloidal crystals (Matsuoka et al., 1996) are clearly inconsistent with the DLVO theory. Some of these unexpected results may be caused by experimental difficulties; for example, Palberg and Würth (1994) presented evidence that the system studied by Tata and co-workers was not at equilibrium. Similar anomalous results have been obtained for colloids in confined systems where metastable colloidal crystallites have been studied (Larsen and Grier, 1997). The structure and dynamics of these crystals show evidence of long-range attractions between similarly charged particles (Larsen and Grier, 1997; Murray, 1997). Theoretical explanations of these experimental results were offered by Sogami and Ise (Sogami, 1983; Sogami and Ise, 1984). The theory has caused controversial responses in this field of science (Levine and Hall, 1992; Overbeek, 1993; Bowen and Sharif, 1998); for detailed review, see Schmitz (1993) and Vlachy (1999). It is quite clear, however, that neither the classical DLVO theory, nor the new approach developed by Sogami and coworkers, is able to explain all these phenomena.

In this paper, we wish to show that anomalous results observed in the experiments mentioned above can be explained in terms of the strong correlation between the counterions caught in the field of a macroion. The idea that the electrostatic interaction that arises from fluctuations in charge could give rise to attractive forces between protein molecules was first proposed by Kirkwood and Shumaker (1952). Later, it was suggested (Oosawa, 1968) that precipitation of rodlike polyions resulting from addition of multivalent ions is caused by ion fluctuations. Similar ideas have been explored by other authors (Ray and Manning, 1994; Gronbech-Jensen et al., 1997). Rouzina and Bloomfield (1996) proposed an electrostatic theory for DNA attraction to explain the condensation of DNA in the presence of multivalent counterions (Bloomfield et al., 1994; Tang et al., 1996). Among theoretical results that support the view that the force between two equally charged surfaces can be attractive are Monte Carlo simulations (Guldbrand et al., 1984; Valleau et al., 1991; Lyubartsev and Nordenskiöld, 1995; Lyubartsev et al., 1998). The simulations are supported by actual measurements of forces between charged mica surfaces immersed in an aqueous electrolyte solution (Kjellander et al., 1990; Kekicheff et al., 1993). The simulation results mentioned above apply to infinitely large charged surfaces or to an array of cylinders immersed in an electrolyte solution, and, therefore, cannot provide any structural information about macroions in solution. In other words, though the computer results presented so far indicate a presence of an attractive force between the equally charged polymeric ions, they do not show actual clustering of the macroions due to this force.

Recently (Hribar and Vlachy, 1997), the Monte Carlo results for solutions of macroions and counterions were reported. The ions were represented as charged hard spheres moving in a continuous dielectric. It was found that the properties of solutions with divalent counterions differ qualitatively from those with monovalent counterions (see also Rebolj et al., 1997). In particular, the presence of divalent counterions in solution causes a nonuniform distribution of macroions; an effect that is clearly not consistent with the DLVO theory. This observation has prompted us to examine the influence of the valency of counterions on the

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interaction between macroions in a more systematic manner. The results are presented below.

## MODEL AND METHOD

In this calculation, the ions were treated as charged hard spheres, and the solvent was considered as a continuum with a dielectric constant  $\epsilon$  equal to that of bulk water at  $T = 298\text{K}$ . In this model, the ions interact via a pairwise additive potential  $u_{ab}$ ,

$$u_{ab}(r) = \begin{cases} \infty, & r < (r_a + r_b) \\ \frac{e^2 z_a z_b}{4\pi\epsilon_0\epsilon r}, & r \geq (r_a + r_b). \end{cases} \quad (1)$$

Here,  $r$  is the distance between the particles, and  $r_a$  is the radius of a particle of type  $a$ . Also,  $z_a$  is the valency of an ion of type  $a$ , and  $e$  is the proton charge. The indices,  $a$  and  $b$ , stand for macroions (p) and counterions (c). The radii of ions in this calculation were  $r_p = 1.0\text{ nm}$  and  $r_c = 0.1\text{ nm}$ , and the corresponding valencies were  $z_p = -12$ , and, for counterions,  $z_c = +1$ ,  $z_c = +2$ , or  $z_c = +3$ .

The Monte Carlo simulations were performed at constant volume and temperature, with 64 (or in some cases 128) macroions and an equivalent number (required by the electroneutrality condition) of counterions in the system. The standard Metropolis algorithm was applied and, to minimize effects due to the small number of particles included in the simulation cell, we used the Ewald summation method (Allen and Tildesley, 1989). In calculating the statistics, much care was exercised with the averages collected over 50 million Monte Carlo moves, after an equilibration run of at least 5 million configurations. Note that the surface charge density of the model macroions is higher than in the  $-20:+1$  ( $-20:+2$ ) cases studied before (Hribar and Vlachy, 1997).

## RESULTS AND DISCUSSION

As already mentioned, all the results presented in this section apply to aqueous solutions at  $298\text{K}$ . The simulation results for the macroion-macroion distribution function,  $g_{pp}(r)$ , are discussed first. Figure 1 displays the results for  $-12:+1$ ,  $-12:+2$ , and  $-12:+3$  solutions at  $c_p = 0.01\text{ mole dm}^{-3}$ . This figure shows that the valency of counterions plays an essential role in determining the macroion-

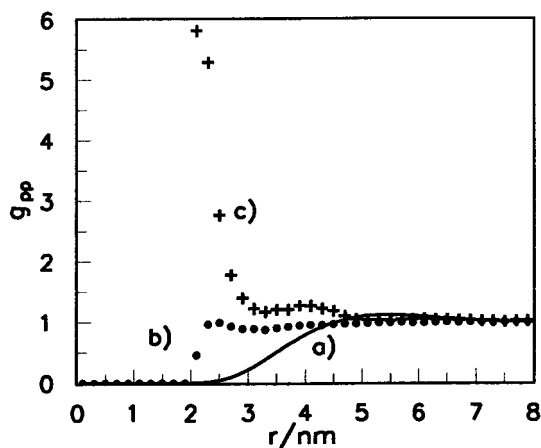


FIGURE 1 The macroion-macroion pair distribution functions at  $c_p = 0.01\text{ mole dm}^{-3}$ . a,  $-12:+1$ ; b,  $-12:+2$ ; and c,  $-12:+3$  electrolytes.

macroion interaction. For solutions with monovalent counterions, the interaction is purely repulsive and the macroions are distributed at large distances from each other. The position of the very broad first peak in  $g_{pp}(r)$  indicates that the highest probability of finding a nearest neighbor is  $\sim r^* = 5.4\text{ nm}$ . The shape of the curve describing the macroion-macroion correlations in  $-12:+2$  solutions is somewhat different. The peak of the pdf is here shifted toward smaller distances ( $r^* = 2.4\text{ nm}$ ); however, the two macroions are not in contact but merely share a layer of counterions. These findings are in agreement with the results of our previous study (cf. Fig. 3 of Hribar and Vlachy, 1997). The third curve, showing the pdf for macroions in solutions with trivalent counterions, is qualitatively different from the other two pdfs. It reflects a high probability of two macroions being in contact, whereas the hump around  $r = 4\text{ nm}$  indicates an increased probability for three macroparticles to form a cluster. This is a counter-intuitive result that is not consistent with the DLVO theory; repulsion is expected between equally charged macroions. In this way, it is possible to explain the precipitation of polyelectrolytes often observed in solutions with multivalent counterions (Olvera de la Cruz et al., 1995; Raspud et al., 1998).

Very interesting is the concentration dependence of the macroion-macroion pdfs. In Fig. 2, we present the three pdfs for  $c_p = 0.005\text{ mole dm}^{-3}$ . The contact value of the macroion-macroion pdf,  $g_{pp}(r)$ , for trivalent counterions is even higher for this concentration. Our simulations for  $-12:+3$  electrolyte solutions, presented in Fig. 3, indicate that  $g_{pp}(r = 2\text{ nm})$  first increases with increasing polyelectrolyte concentration, reaches a maximum, and then decreases upon further increase of  $c_p$ . The contact value of  $g_{pp}(r)$  is below unity for concentrations  $c_p$  smaller than  $0.0001\text{ mole dm}^{-3}$ . The situation is different for solutions containing mono- (or divalent) counterions, where contact values of the macroion-macroion pdf is close to zero in the concentration interval studied here, and they increase slightly with an increasing polyelectrolyte concentration.

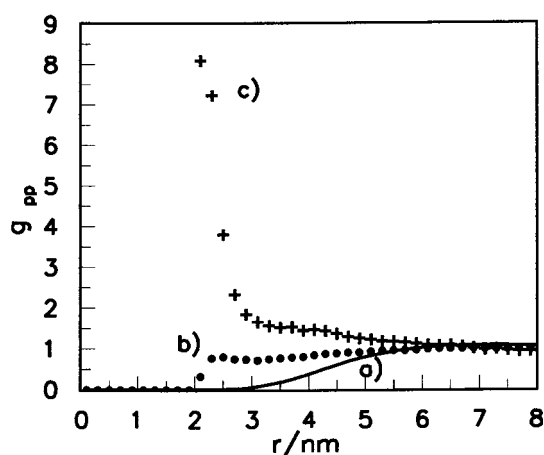


FIGURE 2 The same as for Fig. 1 but at  $c_p = 0.005\text{ mole dm}^{-3}$ .

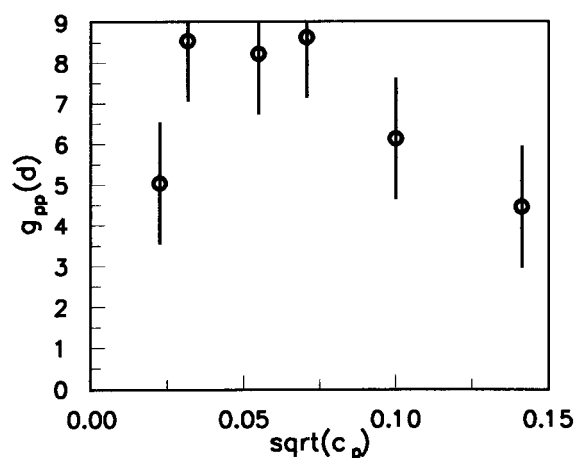


FIGURE 3 The value of macroion-macroion pair distribution function for  $-12:+3$  electrolytes at contact ( $r = d = 2$  nm) as a function of the square root of polyelectrolyte concentration  $c_p$ .

Next, we discuss the counterion-counterion and counterion-macroion pair distribution functions. In Fig. 4, the counterion-counterion pdfs are presented for  $c_p = 0.005$  mole  $\text{dm}^{-3}$ . As expected, the correlation between trivalent ions is much stronger than the correlation between mono- or between divalent counterions. Similar conclusions apply to the counterion-macroion pdf, shown in Fig. 5. This function indicates a high accumulation of multivalent counterions around the macroion; more quantitatively, the values of  $g_{pc}(r = 1.1$  nm) in  $-12:+1$ ,  $-12:+2$ , and  $-12:+3$  solutions at  $c_p = 0.005$  mole  $\text{dm}^{-3}$  are around 29, 120, and 250, respectively. The pdf,  $g_{pc}(r)$ , for the  $12:+3$  solution is different from the corresponding pdfs for the other two solutions; this function exhibits an additional peak located around  $r = 3.1$  nm. We consider this peak as an indirect proof of the partial dimerization of macroions.

Figures 6 and 7 show typical equilibrium arrangements of

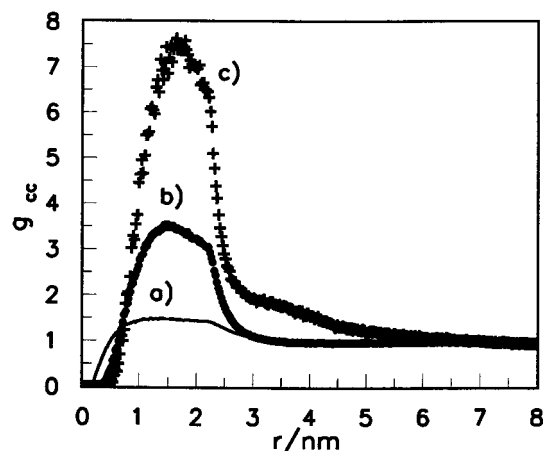


FIGURE 4 The counterion-counterion pair distribution functions at  $c_p = 0.005$  mole  $\text{dm}^{-3}$ . *a*,  $-12:+1$ ; *b*,  $-12:+2$ ; and *c*,  $-12:+3$  electrolytes.

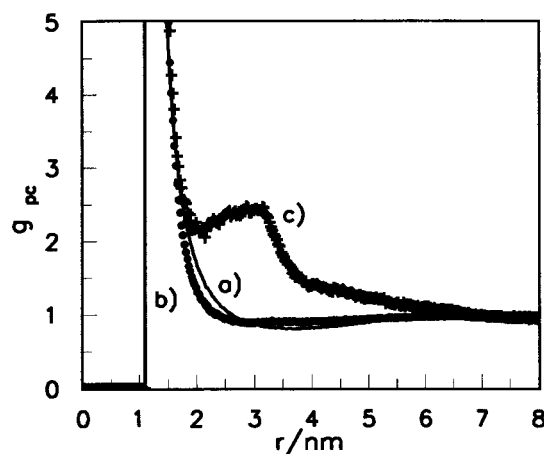


FIGURE 5 The counterion-macroion pair distribution functions at  $c_p = 0.005$  mole  $\text{dm}^{-3}$ . *a*,  $-12:+1$ ; *b*,  $-12:+2$ ; and *c*,  $-12:+3$  electrolytes.

macroions attained in the simulation for two different solutions, one with monovalent and the other with trivalent counterions, both at a concentration  $c_p = 0.005$  mole  $\text{dm}^{-3}$ . The macroions in the  $-12:+1$  electrolyte (Fig. 6) are distributed quite uniformly, and we propose that a cell model might be a good approximation in this case (Rebolj et al., 1997). In Fig. 7, the result for the  $-12:+3$  solution is presented. The pdf for this case, presented in Fig. 2, indicates clustering of macroions, as is actually observed in Fig. 7. Strong interionic correlations yield a nonuniform distribution (clusters of macroions and large voids) of macroparticles in solution. The cell model, often used to interpret experimental results in micellar systems, is clearly inappropriate here. For the sake of simplicity, the counterions are not shown in these figures. Closer inspection of these results reveals a spherically symmetric distribution of counterions

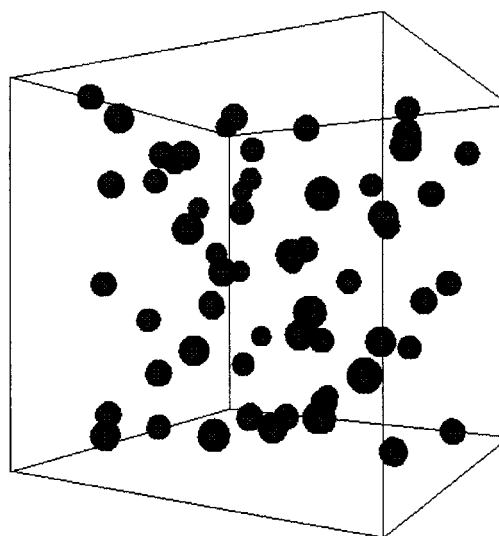


FIGURE 6 An example of equilibrium distribution of macroions for  $-12:+1$  electrolyte at  $c_p = 0.005$  mole  $\text{dm}^{-3}$ .

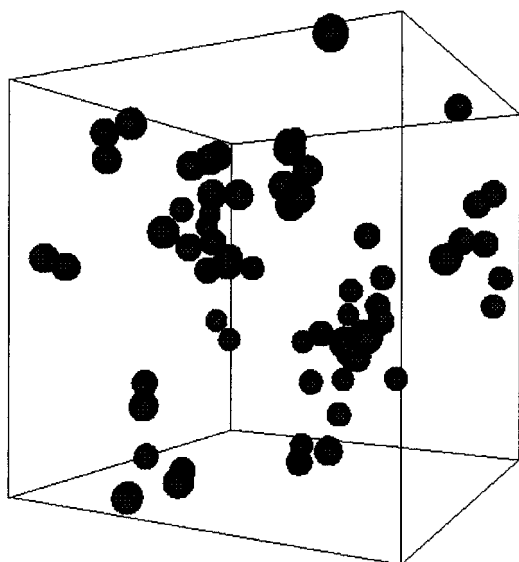


FIGURE 7 The same as for Fig. 6 but for  $-12:+3$  electrolyte.

in the  $-12:+1$  solutions. For  $-12:+2$  solutions, most of the counterions are located in the narrow gap between the two macroions: in solutions with trivalent counterions, the highest probability of finding the counterion is in the wedge-shaped space between the two macroions in contact.

Next, we briefly discuss some thermodynamic properties. The interparticle distributions are reflected in the osmotic coefficient,  $\phi$ , defined as the ratio between the actual and ideal osmotic pressure  $\Pi/\Pi_{\text{ideal}}$ . The osmotic pressure was calculated using the virial equation (Allen and Tildesley, 1989). The values of  $\phi$  for  $-12:+1$ ,  $-12:+2$ , and  $-12:+3$  solutions at a concentration  $c_p = 0.005$  mole  $\text{dm}^{-3}$  are  $0.57 \pm 0.005$ ,  $0.31 \pm 0.01$ , and  $0.14 \pm 0.04$ , respectively. The low value of  $\phi$  in the  $-12:+3$  solution reflects the strong attractive interaction between counterions and macroions and also the clustering of macroions. The structural data presented in Figs. 1 and 2 indicate that solutions with monovalent ions, when subjected to dilution, behave differently from solutions containing multivalent counterions. For this reason, we decided to investigate the concentration dependence of the basic thermodynamic functions. The results for internal energy ( $E$ ), entropy ( $S$ ) and free energy ( $A$ ) differences upon dilution from  $c_p = 0.02$  to  $0.01$  mole  $\text{dm}^{-3}$  are shown in Table 1. Excess internal energies were calculated via the standard thermodynamic equation

**TABLE 1** The internal energy,  $E/Nk_B T$ , entropy,  $S/Nk_B$ , and free energy,  $A/Nk_B T$ , changes upon dilution from  $c_p = 0.02$  to  $0.01$  mole  $\text{dm}^{-3}$

$z_c$	$\Delta E/Nk_B T$	$\Delta S/Nk_B$	$\Delta A/Nk_B T$
+1	$0.260 \pm 0.003$	$0.621 \pm 0.006$	$-0.362 \pm 0.003$
+2	$0.27 \pm 0.01$	$0.36 \pm 0.02$	$-0.10 \pm 0.01$
+3	$0.3 \pm 0.1$	$0.3 \pm 0.2$	$0.0 \pm 0.1$

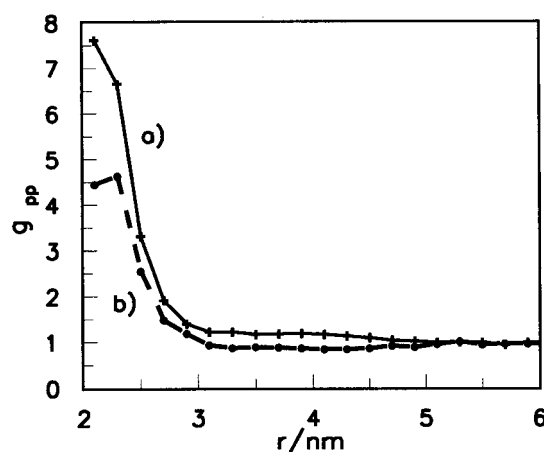


FIGURE 8 The macroion-macroion pair distribution functions for  $-12:+3$  electrolyte at  $c_p = 0.003$  mole  $\text{dm}^{-3}$ . *a*, no added simple electrolyte; *b*, concentration of added  $-1:+3$  electrolyte is  $0.01$  mole  $\text{dm}^{-3}$  (concentration of monovalent co-ions is  $0.03$  mole  $\text{dm}^{-3}$ ).

and the free energy by integration of the Gibbs-Helmholtz equation in the form,

$$\beta^0 A = \beta^0 A^{\text{ideal}} + \int_{\beta=0}^{\beta^0} E^{\text{ex}}(\beta) d\beta, \quad (2)$$

where  $A^{\text{ideal}}$  represents an ideal part of the free energy. Further,  $\beta = 1/k_B T$  ( $k_B$  being the Boltzmann constant and  $T$  is the absolute temperature), whereas  $\beta^0$  applies to  $T = 298\text{K}$ . The small value of the Helmholtz free energy change indicates an energy-entropy compensation for solutions with divalent and trivalent counterions.

According to the DLVO theory, stability against aggregation is a consequence of the repulsive interaction between similarly charged electrical double-layers. Addition of a simple electrolyte causes a compression of the diffuse double-layer around macroparticles and, therefore, destabilizes the solution. Our results (Fig. 8) show that this may not always be true. In particular, an addition of a  $0.01$  M solution of  $-1:+3$  electrolyte (the co-ions and counterions are of equal size) to a  $-12:+3$  polyelectrolyte solution of  $c_p = 0.003$  M decreases the macroion-macroion contact value; moreover, it moves the peak of  $g_{pp}(r)$  from  $r = 2$  nm toward a larger distance ( $2.2$  nm). This interesting finding is in qualitative agreement with an experimental study of the salt concentration dependence of the interparticle distance in colloidal suspensions (Matsuoka et al., 1996).

## CONCLUSIONS

The simulation results presented in this work show unambiguously that strong interionic correlations yield an attraction between equally charged macroions. Ion-ion correlations depend on the charge densities of the counterions and



macroions present in the system and on the dielectric constant of the solvent. The results indicate the limitations of the DLVO, or any other theory that ignores correlations between the ions in the electrical double-layer. There is no need to introduce an additional attractive force to explain the clustering and possible precipitation of macroions. These findings may be of importance for understanding practical problems in the technology of colloidal suspensions and micellar solutions (Murray, 1997). Our calculation suggests a possible explanation for the coexistence of regions dense in macroions and large voids in colloidal suspensions as, also for the polyelectrolyte precipitation in presence of multivalent counterions, observed experimentally. Knowledge of the stability of polyelectrolyte solutions is of considerable importance for the biological sciences. For example, aggregation of proteins is involved in many processes from the food industry and biotechnology to disease states: there is a group of diseases in which a pathological separation into coexisting protein-rich and protein-poor phases takes place. The mechanism of this separation process is not yet clear (Liu et al., 1995).

After this manuscript was ready for submission, the recent paper of Wu et al. (1998) became available to us. These authors studied the potential of the mean force between two macroions (infinite dilution limit) in an electrolyte solution containing divalent counterions. Their study confirmed our previous findings about the attraction between macroions in solutions with divalent counterions (Hribar and Vlachy, 1997).

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